Impact of cow manure and ash (wood ash and charcoal) for reducing lead and arsenic availability and accumulation in *Ipomoea aquatica* L., *Spinacia oleracea* L. and *Amaranthus gangeticus* L.

Ahmad M[1*], Rahman M.S[1], Junayed M[4], Haque M.M[3], Yeasmin N[3], Ahmed T[3], Sarwar N[2] and Akther S[2]

[1]Department of Applied Chemistry and Chemical Technology, Chattogram Veterinary and Animal Sciences University
[2]Department of Food Processing and Engineering, Chattogram Veterinary and Animal Sciences University
[3]Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University
[4]Forest Protection Division, Bangladesh Forest Research Institute

Corresponding Author: Monsur Ahmad, Department of Applied Chemistry and Chemical Technology, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh; E-mail: ahmadmonsurfst@gmail.com

DOI: 10.31364/SCIRJ/v10.i12.2022.P1222938
http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

Abstract

Leafy vegetables are the major dietary source of vitamins, minerals, fibers and bioactive compounds. Due to rapid urbanization and industrialization, an increasing level of heavy metals in vegetables has been noticed in recent years. This study aimed to evaluate the effects of cow manure and ash like materials for reducing arsenic and lead accumulation in three common leafy vegetables. Therefore, a cross-sectional study was conducted on three leafy vegetable samples in Chattogram, Bangladesh. A total of 54 leafy vegetable samples of three species like water spinach, spinach and red amaranth were collected from the six treatment groups which were treated with wood ash, cow manure, charcoal and their mixture and the concentration of arsenic and lead were evaluated by Atomic Absorption Spectrometry (AAS). Results indicated that soil contains a higher level of lead which exceeds the maximum permissible limit followed by WHO. Again, cow manure, wood ash and charcoal significantly reduce the concentration of arsenic and lead in soil. Accumulation of arsenic was significantly reduced by the mixture of cow manure and charcoal in Water Spinach, wood ash and mixture of wood ash and charcoal in Spinach, and wood ash in Red Amaranth. Again, the accumulation of lead was reduced by cow manure, wood ash, charcoal and their mixture, though this reduction was not significant. So, cow manure, wood ash and charcoal have a significant effect on the arsenic accumulation in leafy vegetables because they increase the stability and decrease the mobility of heavy metals in soil.

Keywords: Heavy metal, permissible limit, health risk and AAS.

Introduction

Heavy metals are the metallic chemical element that has a relatively high density (above 3.5 g/cm3 to above 7 g/cm3), toxic or poisonous at low concentration and have a significant impact on the environment (1). Though the major source of heavy metals is the earth's crust, many anthropometric activities like industrial activities, domestic activities, shipbreaking, smelting operations and agricultural use are also important sources (2,3). Rapid urbanization and industrialization are also responsible for heavy metal poisoning (4). These natural and main maid activities drastically pollute the atmosphere, soil and water and finally enter into the food chain. So, the primary sources

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

of heavy metals are water, soil and air from which heavy metals are transmitted to plant and animal bodies (5). Heavy metals enter the human body and bio-accumulate inside their tissues as well as being processed when humans are subjected to pollution, such as by inhalation, absorption through the skin, contaminated food, and drinking water. These heavy metals are subsequently absorbed by plants and animals as well as by human feces. They are transferred from one source to another in this manner (6). Vegetables can accumulate heavy and trace elements from several sources, but the main one is thought to be the soil and water. One of the primary routes through which (heavy/trace) metals enter the human body is through the consumption of vegetables because, in Bangladesh people consume vegetables in every meal of diet. After being ingested, heavy metals are deposited in the fat and bone tissues, displacing noble minerals and leading to a variety of disorders (7). So, continuous intake of heavy metal contaminated vegetables has significant health problem for living body.

Vegetables are an important source of dietary fiber in Bangladesh and per capita average consumed 26 kg/year (8). They are also an important source of vitamins, minerals, trace elements and bioactive compounds which are essential for nutrition and health. Both rural and urban people intake vegetables every meal and it is an important part of their daily diet. This consumption is increasing mostly among urban people day-by-day. But unfortunately, due to the contaminated water and soil and water, these leafy vegetables are drastically polluted by heavy metals. It is also noticed that leafy vegetables have a higher affinity to absorb and accumulate heavy metals compared with other vegetables. When these contaminated vegetables are consumed, they cause adverse health effects in the human body (9). Increased levels of these metals impede plant growth processes like respiration, photosynthesis, water uptake, and nutrient uptake, which lowers plant productivity. Vegetables have been shown to contain higher levels of heavy metal pollution recently, which could have serious consequences for human health (10). Consistent eating of vegetables tainted with heavy metals may result in their buildup in people, disrupting a variety of biochemical processes and resulting in disorders of the heart, brain system, kidneys, and bones (11).

Soil composition, atmospheric conditions and fertilizers are the main sources of heavy metals in vegetables. Again, the use of natural fertilizers like cow manure and ash-like materials like wood ash and charcoal have positive heavy metal binding properties and reduced heavy metal accumulation in plants and vegetables (12). Pb and As are two important heavy metals which cause a drastic public health problem and their accumulation by vegetables from the soil is the main exposure pathway for a human being. The immobilization of Pb and As decreases their solubility and prevents them from being transported and distributed from contaminated soils. To immobilize heavy metals in soil and water, several organic additions are being tested (13). Using cow manure and ash-like ingredients as an alternative to commercial fertilizer increase soil fertility and soil stability (14). Furthermore, it is a low-cost technology without causing negative impacts on soil and crops.

In Bangladesh, several types of vegetables are cultivated in the agricultural field, rail/roadsides and free spaces around the house in both urban and rural areas. Leafy vegetables are more common due to the small cultivating period. Unfortunately, due to the contaminated environment, these vegetables are contaminated by toxic heavy metals. There have been some studies focused on heavy metal-contaminated soil and vegetables. But very few studies have investigated the impact of cow manure and ash-like materials on reducing heavy metal availability and accumulation in vegetables. Therefore, the present study was conducted on three leafy vegetables like Water Spinach (*Ipomoea aquatica* L.), Spinach (*Spinacia oleracea* L.) and Red amaranth (*Amaranthus gangeticus* L.) to identify the impact of cow manure and ash like materials (wood ash, charcoal) on heavy metal accumulation in edible parts of these two leafy vegetables.

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

Materials and methods

Study area and design

This study was conducted on Chattogram, which is the commercial capital of Bangladesh. At first an agricultural land was selected and divided into six blocks. They were identified as blocl-1, block-2, bl0ck-3, block-4, block-5 and block-6. Then the five blocks were treated with wood ash, cow manure, charcoal, the mixture of cow manure and charcoal, mixture of charcoal and wood ash. Block-6 was considered as control. Then, three types of vegetables were cultivated for up to 40 days.

Collection of soil and leafy vegetable samples

A total of 18 soil samples were collected from six blocks of experimental land and transferred to the laboratory. For each sample, the soil was collected from the same place with three different depths like 0 cm, 10 cm and 15 cm, finally allowed for hand mixing and sampling was done from the mixture. Then three matured leafy vegetable samples for each vegetable were randomly collected by hand harvesting process. After harvesting, these samples were washed with distilled water and allowed for sun drying. Finally, 18 soil samples and 54 leafy vegetable samples were transferred to the laboratory by covering them with a simple poly bag.

Digestion of soil and vegetables for heavy metal analysis

Both soil and vegetable samples were digested by a microwave digester. For soil, at first 0.3 g of soil sample was taken in a vessel (Model no XP-1500) of the microwave oven with 2.5 ml of 65% HNO_3 and 7.5 ml of 37% HCl. Then heated at 180°C for 1 min and held for 25 min and finally cooled for 10 min. After digestion, the rotor must be cooled to a temperature of 60 °C. Once cooled, the upper screw of the vessel must be carefully undone with a torque wrench to release pressure within a fume hood. For, vegetable sample, 0.5 g grinded mixture of leaf and stem were taken with 5 ml of 65% HNO_3, 1 ml of 37% HCl and 1 ml of 55% $HClO_4$ in a vessel (Model no XP-1500) of the microwave oven. Then heated at 150 °C with a ramping time of 1 min, holding time of 20 min and cooling time of 10 min. The resulting samples were collected, filtered, and leveled with deionized water in a 10 ml volumetric flask. Finally, both soil and vegetable samples were collected, filtered and leveled with deionized water in a 10 ml volumetric flask (16).

Analysis of heavy metal by AAS

Pb and As concentrations were analyzed by Atomic Absorption Spectrophotometer (AAS), Model: ZEEnit 700P, Germany. At first digested leafy vegetables and soil samples were allowed for filtration through Whatman filter paper (0.45 µm pore size) in a 100 ml round bottle flask. Then added deionized water up to the mark and stored as stock solution for AAS. Then 12 ml sample was taken into the falcon tube from stock solution and used for analysis by AAS (17).

Statistical analysis

For statistical analysis, data were exported from Microsoft Excel 2010 and stored there before being entered into STATATM 11.0 (Stata Corporation, College Station, TX, USA). Utilizing percentages, means, and standard deviation for various variables, descriptive analysis was carried out. Finally, to compare the amount of heavy metal residues in leafy vegetables, a one-way ANOVA and post-hoc test was utilized. The level of significance was set ≤ 0.05.

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

Result and discussion

Concentrations of As and Pb in soil

As concentration in soil and three types of leafy vegetables are shown in Table-1. The minimum concentration was 8.68 ± 0.14 mg/kg in block-2 which was treated with cow manure and maximum concentration was 11.87 ± 0.57 mg/kg in control group. The overall concentration of As in six blocks of the field are below the maximum permissible limit followed by World Health Organization (WHO) and Food and Agricultural Organization (FAO) (18). Concentration of Pb in soil and three leafy vegetables are shorn in Table-2. The minimum concentration of Pb was 14.18 ± 0.22 mg/kg in block-1 and maximum concentration was 22.47 ± 0.42 mg/kg in block-6 which was considered as control. Concentration of Pb in soil exceed the maximum permissible limit followed by WHO (19).

Table-1: Average concentration of As in soil and three green leafy vegetables

Samples	Block-1 (N=18)	Block-2 (N=18)	Block-3 (N=18)	Block-4 (N=18)	Block-5 (N=18)	Block-6 (N=18)	p (1-ANOVA)	Maximum permissible limit (mg/kg)
Water Spinach	0.06 ± 0.05	0.27 ± 1.11	0.42 ± 0.54	ND	0.29 ± 0.26	0.43 ± 1.12	0.005	0.50
Spinach	ND	0.27 ± 0.44	0.15 ± 0.14	0.29 ± 0.41	ND	0.48 ± 0.11	0.083	0.50
Red Amaranth	ND	0.19 ± 0.77	0.34 ± 0.34	0.12 ± 0.80	0.16 ± 0.45	0.26 ± 1.15	0.044	0.50
Soil	10.21 ± 0.57	8.68 ± 0.14	10.68 ± 0.27	11.51 ± 0.29	9.85 ± 0.33	09.87 ± 0.57	0.205	11.75

Here, block-1 is wood ash, block-2 is cow manure, block-3 is charcoal, block-4 is mixture of cow manure and charcoal, block-5 is mixture of wood ash and charcoal, block-6 is control, ND= not detectable.

For both As and Pb concentrations were maximum in control group than other blocks which were treated by cow manure, wood ash, charcoal and their mixture. It indicates that, when soil is treated with natural remediation, it reduces the mobility of heavy metal and also increase the soil stability. This finding is also supported by others previous studies (20, 21). In six blocks of the treatment group, the overall prevalence of Pb was 100% (N=18). Every block has a greater Pb concentration than As, and this conclusion is consistent with earlier research (22). The prevalence of industrial waste, leaded gasoline, and other anthropogenic causes in the Chittagong region are to blame for the high concentration of Pb. This same finding was also stated by others (23, 24).

Concentration of As and Pb in water spinach

Concentration of As and Pb of Water Spinach are shown in Table-1 and Table-2. All of the Water Spinach samples contain As within safe limit. Control block contains maximum level (0.43 ± 1.12 mg/kg) and block-4 had minimum level. So, all the organic supplement has significant effect to reduce As accumulation in leafy vegetables. In water spinach sample, the concentration of Pb in six treatment groups were in control (2.31 ± 1.08 mg/kg), wood ash (1.65 ± 1.5 mg/kg), cow manure (1.05 ± 0.99 mg/kg), charcoal (1.04±0.99 mg/kg), mixture of cow manure and charcoal (1.14 ± 0.96 mg/kg) and mixture of wood ash and charcoal (1.59 ± 0.67 mg/kg). As concentrations in Water Spinach samples exist within the permissible limit and Pb exceeded the maximum tolerable limit followed by WHO (18). This result is also similar with some previous study (25). Though, organic supplements were significantly reducing the concentration Pb in Water Spinach although these supplements are not sufficient to reduce the accumulation of Pb in water spinach. Higher prevalence of Pb might be due to the heavy metal contaminated soil and water in agricultural field. Because plants absorb a number of elements from soil, some of which have no known biological function and some are known to be toxic at low concentrations (26).

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

Concentration of As and Pb in Spinach

In Spinach sample, As concentrations were in cow manure (0.27 ± 0.44 mg/kg), charcoal (0.15 ± 0.14 mg/kg), mixture of cow manure and charcoal (0.29 ± 0.41 mg/kg) and control (0.48 ± 0.11 mg/kg). In block-1 and block-5 which contain wood ash and the mixture of wood ash and charcoal, As were not detected. As concentration in Spinach samples were within safe limit and this is due to the low concentration of As in soil and water (18). It was also observed that wood ash and the mixture of wood ash and charcoal have significant effects of As reduction in Spinach sample. Here, As content is not significantly increased or decreased in six treatment groups with control. Pb concentration of Spinach is shown in Table-2. It is shown that, Pb content is prevenient in all Spinach sample though experimental blocks has low level of Pb than control block. Concentration of Pb in Spinach is higher than previous study that should be a public health concern.

Table-2: Average concentration of Pb in soil and three green leafy vegetables

Samples	Block-1 (N=18)	Block-2 (N=18)	Block-3 (N=18)	Block-4 (N=18)	Block-5 (N=18)	Block-6 (N=18)	p (1-ANOVA)	Maximum permissible limit (mg/kg)
Water Spinach	1.65 ± 1.5	1.05 ± 0.99	1.04 ± 0.99	1.14 ± 0.96	1.59 ± 0.67	2.31 ± 1.08	0.006	0.30
Spinach	1.07 ± 1.74	1.18 ± 1.94	1.72 ± 2.82	1.42 ± 2.16	1.05 ± 4.11	2.19 ± 3.89	0.007	0.30
Red Amaranth	1.57 ± 0.44	2.37 ± 0.88	2.49 ± 0.30	1.97 ± 0.43	1.54 ± 1.44	3.46 ± 3.00	0.422	0.30
Soil	14.36 ± 0.22	16.58 ±0.17	18.99 ± 0.81	18.75 ± 0.25	19.90 ± 0.26	22.47 ± 0.42	0.004	10.00

Here, block-1 is wood ash, block-2 is cow manure, block-3 is charcoal, block-4 is mixture of cow manure and charcoal, block-5 is mixture of ash and charcoal, block-6 is control, ND= not detectable.

Concentration of As and Pb in Red Amaranth

In Red Amaranth, concentration of As in six experimental groups were block-1 (ND), block-2 (0.19 ± 0.77 mg/kg), block-3 (0.34 ± 0.34 mg/kg), block-4 (0.12 ± 0.80 mg/kg), block-5 (0.16 ± 0.45 mg/kg) and block-6 (0.26 ± 1.15 mg/kg) which is presented in table-1. As concentration was not exceed maximum tolerable limit followed by WHO (18). Compare with other two vegetables, Red Amaranth contains low level of arsenic in all experimental blocks. It might be due to the lower affinity od As with Red Amaranth. Concentration of Pb in Red Amaranth is shown in table-2. It is shown that Red Amaranth contains significant amount of Pb in all samples collected from six blocks. Again, in these vegetables Pb concentrations were higher than As and this is supported by other study (27).

Conclusions

The locality, the soil type, and the method used to handle industrial waste all posed considerable risks for the presence of heavy metals in green leafy vegetables. Greater amounts of heavy metal were present in urban areas than in other regions. It is caused by the pollution of industrial waste, agricultural chemical fertilizers, and waste from sewage systems in towns and cities. This result is consistent with a number of earlier studies (27).

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

References

[1] Tchounwou, P. B. (2018). Heavy metal toxicity and the environment, in Molecular, clinical and environmental toxicology. Journal of Analytical & Bioanalytical Techniques.

[2] Goyer, R. A., & Clarkson, T. W. (1996). Toxic effects of metals. Casarett and Doull's toxicology: the basic science of poisons, 5, 691-736.

[3] Wintz, H., Fox, T., & Vulpe, C. (2002). Functional genomics and gene regulation in biometals research. Biochem. Soc. Transactions, 30(1), 166-168.

[4] Martin, J.A.R., Arana, C.D., Ramos-Miras, J.J., Gil, C., & Boluda, R. (2015). Impact of 70 years urban growth associated with heavy metal pollution. Environmental Pollution, 196, 156–163.

[5] Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qiu, Y., & Liang, J.Z. (2004). Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environment International, 30(6), 785-791.

[6] Ahmad, M., Roy, S. P. K., Sarwar, N., Morshed, S., Alam, M. K., Matin, A., & Kobra, K. T. (2016). Contamination of raw fresh milk, market pasteurized milk and powdered milk by toxic heavy metals in Bangladesh. Scientific Research Journal (SCIRJ), 4(2), 19-24.

[7] Abir, M. H., & Ahmad, M. (2021). Phytochemical, Nutritional and Pharmacological Potentialities of Amaranthus spinosus Linn.: A review. Archives of Ecotoxicology, 3(2), 49-59.

[8] Sarwar, N., Ahmed, T., & Ahmad, M. (2019). Bioactive compounds and antioxidant activity of common vegetables and spices available in Bangladesh. Advance Journal of Food Science and Technology, 17(3), 43-47.

[9] Jolly, Y. N., Islam, A., & Akbar, S. (2013). Transfer of metals from soil to vegetables and possible health risk assessment. SpringerPlus, 2(1), 1-8.

[10] Alia, N., Sardar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., & Toqeer, A. (2015). Toxicity and bioaccumulation of heavy metals in spinach (Spinacia oleracea) grown in a controlled environment. International Journal of Environmental Research and Public Health, 12(7), 7400-7416.

[11] Pan, X. D., Wu, P. G., & Jiang, X. G. (2016). Levels and potential health risk of heavy metals in marketed vegetables in Zhejiang, China. Scientific reports, 6(1), 1-7.

[12] Gul, S., Naz, A., Fareed, I., & Irshad, M. (2015). Reducing heavy metals extraction from contaminated soils using organic and inorganic amendments–a review. Polish Journal of Environmental Studies, 24(3), 1423-1426.

[13] Park, J. H., Choppala, G. K., Bolan, N. S., Chung, J. W., & Chuasavathi, T. (2011). Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant and soil, 348(1), 439-451.

[14] Verheijen, F., Jeffery, S., Bastos, A. C., Van der Velde, M., & Diafas, I. (2010). Biochar application to soils. A critical scientific review of effects on soil properties, processes, and functions. EUR, 24099, 162.

[15] Singh, B., Singh, B. P., & Cowie, A. L. (2010). Characterisation and evaluation of biochars for their application as a soil amendment. Soil Research, 48(7), 516-525.

[16] Kasar, S., Murugan, R., Arae, H., Aono, T., & Sahoo, S. K. (2020). A microwave digestion technique for the analysis of rare earth elements, thorium and uranium in geochemical certified reference materials and soils by inductively coupled plasma mass spectrometry. Molecules, 25(21), 5178.

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938

[17] Lyra, F. H., Carneiro, M. T. W. D., Brandao, G. P., Pessoa, H. M., & de Castro, E. V. R. (2009). Direct determination of phosphorus in biodiesel samples by graphite furnace atomic absorption spectrometry using a solid sampling accessory. Journal of Analytical Atomic Spectrometry, 24(9), 1262-1266.

[18] Abt, E., Fong Sam, J., Gray, P., & Robin, L. P. (2018). Cadmium and lead in cocoa powder and chocolate products in the US Market. Food Additives & Contaminants: Part B, 11(2), 92-102.

[19] Savaci, g., & Oksüz, ç. (2020). Investigation of heavy metal concentrations in soil caused by wild storage dumpsite in kastamonu city. Turkish journal of forest science, 4(1), 26-39.

[20] Beesley, L., Marmiroli, M., Pagano, L., Pigoni, V., Fellet, G., Fresno, T., ... & Marmiroli, N. (2013). Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (Solanum lycopersicum L.). Science of the Total Environment, 454, 598-603.

[21] Kiran, Y. K., Barkat, A., CUI, X. Q., Ying, F. E. N. G., PAN, F. S., Lin, T. A. N. G., & YANG, X. E. (2017). Cow manure and cow manure-derived biochar application as a soil amendment for reducing cadmium availability and accumulation by Brassica chinensis L. in acidic red soil. Journal of integrative agriculture, 16(3), 725-734.

[22] Mottalib, M. A., & Somoal, S. H. (2016). Md contaminated soil and vegetables of tannery area in Dhaka. Bangla Article History: Received 26th February.

[23] Khillare, P. S., Balachandran, S., & Meena, B. R. (2004). Spatial and temporal variation of heavy metals in atmospheric aerosol of Delhi. Environmental Monitoring and Assessment, 90(1), 1-21.

[24] Al-Masri, M. S., Al-Kharfan, K., & Al-Shamali, K. (2006). Speciation of Pb, Cu and Zn determined by sequential extraction for identification of air pollution sources in Syria. Atmospheric Environment, 40(4), 753-761.

[25] Göthberg, A., Greger, M., & Bengtsson, B. E. (2002). Accumulation of heavy metals in water spinach (Ipomoea aquatica) cultivated in the Bangkok region, Thailand. Environmental Toxicology and Chemistry: An International Journal, 21(9), 1934-1939.

[26] Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. The international journal of biochemistry & cell biology, 41(8), 1665-1677.

[27] Islam, M. S., Ahmed, M. K., Raknuzzaman, M., Habibullah-Al-Mamun, M., & Islam, M. K. (2015). Heavy metal pollution in surface water and sediment: a preliminary assessment of an urban river in a developing country. Ecological indicators, 48, 282-291.

http://dx.doi.org/10.31364/SCIRJ/v10.i12.2022.P1222938